Short et al.

Application No.: 09/975,036

Filed: October 10, 2001

Page 2

Attorney Docket No.: DIVER1280-17

PATENT

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Upon entry of the present amendment, the claims will stand as follows:

Please cancel claims 25, 26, 28, 33 and 47-211 without prejudice.

Please amend claims 1, 11-14, 20, 21, 27, 29, 31, 32, 34, 35 and 37 as follows:

- 1. (Currently Amended) A method for identifying a <u>naturally occurring</u> polynucleotide <u>of</u> <u>interest</u> in a liquid phase comprising:
- a) contacting a plurality of <u>naturally occurring</u> polynucleotides derived from at least one organism with at least one nucleic acid probe <u>that comprise a sequence complementary to a bioactivity or biomolecule of interest</u> under conditions that allow hybridization of the probe to [[the]]polynucleotides having complementary sequences of interest, wherein the probe is labeled with a detectable molecule; and
- b) identifying a <u>naturally occurring</u> polynucleotide of interest with an analyzer that detects the detectable molecule a polynucleotide to which a probe has hybridized.
- 2. (Original) The method of claim 1, wherein the polynucleotides are from a mixed population of cells.
- 3. (Original) The method of claim 1, wherein the polynucleotides are in a library.
- 4. (Original) The method of claim 3 wherein the library is an expression library.
- 5. (Original) The method of claim 3 wherein the library is an environmental expression library.
- 6. (Original) The method of claim 1, wherein the nucleic acid probe is from at least about 15 bases to about 100 bases.

In re Application of: Short et al.

Application No.: 09/975,036

Filed: October 10, 2001

Page 3

7. (Original) The method of claim 1, wherein the nucleic acid probe is from at least about 100 bases to about 500 bases.

PATENT

Attorney Docket No.: DIVER1280-17

- 8. (Original) The method of claim 1, wherein the nucleic acid probe is from at least about 500 bases to about 1,000 bases.
- 9. (Original) The method of claim 1, wherein the nucleic acid probe is from at least about 1,000 bases to about 5,000 bases.
- 10. (Original) The method of claim 1, wherein the nucleic acid probe is from at least about 5,000 bases to about 10,000 bases.
- 11. (Currently Amended) The method of claim 1, wherein the detectable molecule is probe is labeled with a fluorescent molecule and the detecting involves detecting fluorescence from the fluorescent molecule from a polynucleotide to which the probe has hybridized.
- 12. (Currently Amended) The method of claim 1, wherein the detectable molecule is probe is labeled with a magnetic molecule and the detecting involves detecting a magnetic field of a polynucleotide to which the probe has hybridized.
- 13. (Currently Amended) The method of claim 1, wherein the detectable molecule hybridization modulates a magnetic field and the analyzer detects modulation of the magnetic field.
- (Currently Amended) The method of claim 1, wherein the detectable molecule 14. hybridization modulates the dielectric signature of the elone-the polynucleotide to which a probe has hybridized

Short et al.

Application No.: 09/975,036

Filed: October 10, 2001

Page 4

15. (Original) The method of claim 1, wherein the analyzer is a FACS analyzer.

- 16. (Original) The method of claim 1, wherein the analyzer is a magnetic field sensing device.
- 17. (Original) The method of claim 9, wherein the magnetic field sensing device is a Super Conducting Quantum Interference Device.

PATENT

Attorney Docket No.: DIVER1280-17

- 18. (Original) The method of claim 1, wherein the analyzer is a multipole coupling spectroscopy device.
- 19. (Original) The method of claim 1, wherein the organism is from an environmental sample.
- 20. (Currently Amended) The method of claim [[-]]1, wherein the environmental sample is selected from the group consisting of[[:]] geothermal fields, hydrothermal fields, acidic soils, sulfotara mud pots, boiling mud pots, pools, hot-springs, geysers, marine actinomycetes, metazoan, endosymbionts, ectosymbionts, tropical soil, temperate soil, arid soil, compost piles, manure piles, marine sediments, freshwater sediments, water concentrates, hypersaline sea ice, super-cooled sea ice, arctic tundra, Sargosso sea, open ocean pelagic, marine snow, microbial mats, whale falls, springs, hydrothermal vents, insect and nematode gut microbial communities, plant endophytes, epiphytic water samples, industrial sites and ex situ enrichments.
- 21. (Currently Amended) The method of claim [[-]]2, wherein the environmental sample is selected from the group consisting of [[:]] eukaryotes, prokaryotes, myxobacteria (epothilone), air, water, sediment, soil or rock.
- 22. (Original) The method of claim 1, wherein the organism comprises a microorganism.

Short et al.

Attorney Docket No.: DIVER1280-17

PATENT

Application No.: 09/975,036 Filed: October 10, 2001

Page 5

- 23. (Original) The method of claim 19, wherein the environmental sample contains extremophiles.
- 24. (Original) The method of claim 23, wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.
- 25. (Cancelled)
- 26. (Cancelled)
- 27. (Currently Amended) The method of claim [[26]] 212, wherein the microenvironment microdroplet is a gel microdroplet.
- 28. (Cancelled)
- 29. (Currently Amended) The method of claim [[28]]212, wherein the biotinylated substrate comprises a core fluorophore structure, a spacer connected to the fluorophore structure by a first connector and connected to the bioactivity or biomolecume of interest microdroplet by a second connector, and two functional groups, wherein each functional group determines specificity of the substrate for an enzyme of interest and is selected from straight and branched alkanes, mono- and oligosaccharides, unsaturated hydrocarbons and aromatic groups.
- 30. (Original) The method of claim 29, wherein the fluorophore is selected from the group consisting of coumarins, resorufins and xanthenes.
- 31. (Currently Amended) The method of claim 29, wherein the spacer is selected from the group consisting of[[:]] alkanes, and oligoethyleneglycols.

Short et al.

Application No.: 09/975,036

Filed: October 10, 2001

Page 6

32. (Currently Amended The method of claim 29, wherein the connector units are selected from the group[[s]] of functional moieties consisting of ether, amine, amide, ester, urea,

PATENT

Attorney Docket No.: DIVER1280-17

and thiourea and other moieties.

33. (Cancelled)

34. (Currently Amended) The method of claim 25212, wherein the analyzer is a flow

cytometer.

35. (Currently Amended) The method of claim [[28]]212, wherein the biotinylated substrate

comprises a core fluorophore structure, a spacer connected to the fluorophore structure by

a first connector and connected to the bioactivity or biomolecule of interest microdroplet

by a second connector, and a quencher component, attached to the cluorophore

fluorophore by a polymer.

36. (Original) The method of claim 35, wherein the fluorophore is selected from the group

consisting of acridines, coumarins, fluorescein, rhodamine, BOPIDY, resorufin, and

porphyrins.

37. (Currently Amended) The method of claim 35, wherein the quencher component is a

moiety capable of quenching fluorescence of the fluorophore.

38. (Original) The method of claim 35, wherein the polymer is selected from the group

consisting of amines, ethers, esters, amides, peptides and oligosaccharides.

39. (Original) The method of claim 35, wherein the spacer is selected from the group

consisting of: alkanes, and oligoethyleneglycols.

Gray Cary\GT\6388272.2 104703-135

PATENT Attorney Docket No.: DIVER1280-17

Short et al. Application No.: 09/975,036

Filed: October 10, 2001

Page 7

- 40. (Original) The method of claim 35, wherein the first and second connectors are selected from the groups consisting of ether, amine, amide, ester, urea, thiourea and other moieties.
- 41. (Original) The method of claim 1, wherein the polynucleotide of interest encodes an enzyme.
- 42. (Original) The method of claim 41, wherein the enzyme is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, eposize hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.
- 43. (Original) The method of claim 1, wherein the polynucleotide of interest encodes a small molecule.
- 44. (Original) The method of claim 1, wherein the polynucleotide of interest, or fragments thereof, comprise one or more operons, or portions thereof.
- 45. (Original) The method of claim 44, wherein the operons, or portions thereof, encodes a complete or partial metabolic pathway.
- 46. (Original) The method of claim 44, wherein the operons or portions thereof encoding a complete or partial metabolic pathway encodes polyketide syntheses.

Claims 47-211. (Cancelled)

Short et al.

Application No.: 09/975,036

Filed: October 10, 2001

Page 8

Please add new claims 212-216 as follows:

212. (New) A method for identifying a polynucleotide of interest comprising:

a) encapsulating in microdroplets a plurality of clones comprising naturally

PATENT

Attorney Docket No.: DIVER1280-17

occurring polynucleotides derived from at least one organism and a biotinylated substrate that

fluoresces upon contact with a specific enzyme, wherein the substrate is attached to the

microdroplet via a biotin-avidin-biotin bridge;

b) incubating the microdroplets under conditions that allow expression of at least

some of the polynucleotides within the microdroplets; and

c) identifying a clone comprising a naturally occurring polynucleotide of interest

with an analyzer that detects fluorescence of the substrate within a microdroplet upon contact of

the substrate with the specific enzyme.

213. (New) The method of claim 212, wherein the polynucleotide of interest encodes a small

molecule.

214. (New) The method of claim 212, wherein the polynucleotide of interest, or fragments

thereof, comprise one or more operons, or portions thereof.

215. (New) The method of claim 214, wherein the operons, or portions thereof, encodes a

complete or partial metabolic pathway.

216. (New) The method of claim 214, wherein the operons or portions thereof encoding a

complete or partial metabolic pathway encodes polyketide syntheses.

Gray Cary\GT\6388272.2 104703-135